H A Z L E T O N
LABORATORIES AMERICA, INC.
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MULTIDOSE RANGE-FINDING STUDY IN RATS

A80

FINAL REPORT

Submitted to

Lorillard
Greensboro, North Carolina

September 9, 1985

HAZLETON
LABORATORIES AMERICA, INC.
9200 LEESBURG TURNPIKE, VIENNA, VIRGINIA 22180, U.S.A.

OFFICE OF QUALITY ASSURANCE

Project Title: Multidose Range-Finding Study

Project No.: 642-268

Quality Assurance review of the final report was conducted according to the procedures described in the standard operating procedures of the Report Review Section of the Office of Quality Assurance, and according to the general requirements of the Good Laboratory Practice regulations that were issued on December 22, 1978, by the Food and Drug Administration for compliance on and after June 20, 1979. The final report review was conducted and the findings were reported to management and to the study director on the following dates:

Final Report Review	Findings Reported	Reviewer
Review 9/2,3/85	9/5/85	P. Miller

Frederick G. Snyder
Director
Office of Quality Assurance

9/3/85

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SUBJECT: Multidose Range-Finding Study in Rats
Project No. 642-268

We, the undersigned, hereby declare that the work was performed under our supervision, according to the procedures herein described.

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SPONSOR: Lorillard DATE: September 9, 1985

MATERIAL: A80

SUBJECT: FINAL REPORT
Multidose Range-Finding Study in Rats
Project No. 642-268

SUMMARY

The test material, A80, was evaluated for the maximum tolerated dose (MTD) level in male and female rats. Based upon the findings of this study, the MTD in male and female rats was estimated to be 1375 mg/kg of body weight.

INTRODUCTION

This study was designed to determine the maximum tolerated dose (MTD) level of A80 following repeated daily oral doses to rats for four days. The Single Dose Range-Finding Study was initiated on June 27, 1985 and was terminated on July 1, 1985. The Multidose Range-Finding Study was initiated on July 30, 1985 and was terminated on August 3, 1985.

TEST MATERIAL

The test material, A80, a white solid, was received from the sponsor on May 17, 1985, and was stored in an amber jar under

refrigeration. Corn oil (Duke's® Pure Corn Oil, The C. F. Sauer Company, Lot No. 52500), a yellow liquid received on April 30, 1985, and stored at room temperature, was used as the vehicle. A value of 100% active ingredient was assumed for purposes of dosage calculations. Information on stability and methods of synthesis, as well as data on composition or other characteristics which define the test material are on file with the sponsor.

TEST ANIMALS

Sprague-Dawley rats were received from Charles River Breeding Laboratories, Inc., Kingston, New York. Animals were randomly housed upon receipt via computer generated random numbers and subsequently assigned to this study following the acclimation period. Two rats of each sex were assigned to the Single Dose Range-Finding Study and six rats of each sex to the Multidose Range-Finding Study and subsequently to groups. The initial body weights of the males ranged from 154.3 to 162.4 grams, and the initial body weights of the females ranged from 117.4 to 136.4 grams in the Single Dose Range-Finding Study. In the Multidose Range-Finding Study, the initial body weights of the males ranged from 182.4 to 224.1 grams, and the initial body weights of the females ranged from 124.3 to 156.5 grams.

The rats were identified uniquely by ear tags and housed individually in elevated wire-mesh cages. Commercial rodent ration (Purina Rodent Laboratory Chow® #5001) and tap water were avail-

able ad libitum except where noted otherwise. During the Single Dose Range-Finding Study, the temperature and humidity in the animal room ranged from 72-74°F and 60-74%, respectively. In the Multidose Range-Finding Study, the temperature in the animal room was 72°F, the humidity ranged from 60-78%. A 12-hour light/dark cycle was maintained daily. The rats were acclimated to laboratory conditions for a minimum of one week prior to initiation of treatment. Rats were used in this study because they have historically been used in safety evaluation studies and are required by appropriate regulatory agencies.

METHODS

Groups and Dosage Levels

Single Dose Range-Finding Study

Two animals of each sex were assigned to the following group as shown below.

Group	No of Animals		Dosage Level mg/kg
	Males	Females	
1	2	2	5000

Multidose Range-Finding Study

Two animals of each sex were assigned to each of the following groups as shown on the following page.

Group	No of Animals Males	Females	Dosage Level mg/kg
1	2	2	500
2	2	2	1000
3	2	2	2500

Compound Preparation and Administration

The test material was first warmed in a water bath at 45-50°C until a liquid state was achieved. The required amount of compound for each level was then weighed into pre-calibrated beakers on an appropriate electronic balance. Corn oil was added to each beaker to bring the mixture to the desired volume. The mixtures were stirred for approximately five minutes on a magnetic stirrer and were stirred in a 40-45°C water bath while dosing. During the Multidose Range-Finding phase, the test material mixtures were stored under refrigeration.

Each rat received the appropriate amount of test material by gavage. Single Dose Range-Finding animals received one dose and Multidose Range-Finding animals received one dose daily for four consecutive days. The test material was administered by gavage because of the characteristics of the test compound.

Observations and Records

Single Dose Range-Finding Study

All of the rats were observed for signs of toxic and pharmacologic effects at one and six hours postdose, and at least once daily thereafter to Day 5. Mortality/moribundity was recorded twice daily. Individual body weights were recorded immediately prior to treatment.

Multidose Range-Finding Study

All of the rats were observed for signs of toxic and pharmacologic effects at one and five hours postdose on Day 1, at one and six hours postdose on Days 2-4, and on Day 5 (24 hours following the Day 4 dose). Mortality/moribundity was recorded twice daily. Individual body weights were recorded immediately prior to the first dose.

Sacrifice and Gross Pathology

Single Dose Range-Finding Study

At termination (Day 5), all surviving rats were sacrificed by carbon dioxide asphyxiation and discarded.

Multidose Range-Finding Study

At termination (Day 5), all surviving rats were sacrificed by carbon dioxide asphyxiation and discarded.

Raw Data and Final Report Storage

All raw data and the final report are stored in the archives of Hazleton Laboratories America, Inc.

RESULTS

Dosing Volumes

Individual dosing volumes for the Single Dose and Multidose Range-Finding Studies are presented in Tables 1A and 1B, respectively.

Mortality

Single Dose Range-Finding Study

No deaths occurred during the Single Dose Range-Finding Study.

Multidose Range-Finding Study

No deaths occurred during the Multidose Range-Finding Study.

Clinical Observations and Body Weights

Single Dose Range-Finding Study

A summary of clinical observations is presented in Table 2A. Initial body weights are presented in Table 3A.

A variety of commonly noted clinical signs was observed in all rats during the study.

Multidose Range-Finding Study

A summary of clinical observations is presented in Table 2B. Initial body weights are presented in Table 3B.

Clinical signs noted in Groups 1-2 were limited to isolated instances of urine stains and soft feces. A variety of commonly noted clinical signs was observed in all Group 3 rats.

Conclusions

Based upon the presence of two incidences of gross toxicity (slight depression and/or depression) in both male and female rats dosed at 2500 mg/kg, the MTD in male and female rats was estimated to be 1375 mg/kg of body weight.

Table 1A
Dosing Volumes
Multidose Range-Finding Study in Rats
Single Dose Range-Finding Study

Animal Number	Group	Sex	Dosage Level mg/kg	Dosage Volume ml
D96747	1	M	5000	1.5
D96748	1	M	5000	1.6
D96753	1	F	5000	1.4
D96754	1	F	5000	1.2

Table 1B
Dosing Volumes*
Multidose Range-Finding Study in Rats
Multidose Range-Finding Study

Animal Number	Group	Sex	Dosage Level mg/kg	Dosage Volume ml
D96983	1	M	500	7.2
D96984	1	M	500	8.8
D96995	1	F	500	5.2
D96996	1	F	500	6.0
D96985	2	M	1000	7.6
D96986	2	M	1000	7.6
D96997	2	F	1000	6.4
D96998	2	F	1000	5.2
D96987	3	M	2500	8.0
D96988	3	M	2500	8.8
D96999	3	F	2500	6.0
D97000	3	F	2500	4.8

* The dosage volume for each animal is a total of four equal doses.

Table 2A
Summary of Clinical Observations*
Multidose Range-Finding Study in Rats - Single Dose Range-Finding Study
Group 1 - 5000 mg/kg

OBSERVATION	Observation Intervals: Day 1		Day 2		Day 3		Day 4		Day 5	
	1 Hour	6 Hours	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Males										
Normal	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	2/2	2/2
Prostrate	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Depression	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Slight depression	0/2	2/2	2/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Labored respiration	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Rough coat	1/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	0/2	0/2
Urine stains	1/2	1/2	1/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2
Females										
Normal	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	1/2	1/2
Prostrate	2/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Depression	0/2	0/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Slight depression	0/2	1/2	1/2	1/2	1/2	0/2	0/2	0/2	0/2	0/2
Ataxia	0/2	0/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Labored respiration	1/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Soft feces	0/2	1/2	1/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2
Rough coat	0/2	1/2	2/2	2/2	2/2	2/2	2/2	2/2	0/2	0/2
Urine stains	0/2	1/2	2/2	2/2	1/2	1/2	1/2	1/2	1/2	1/2
Red stains on nose and/or eyes	0/2	0/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Hunched	0/2	0/2	1/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2

* Numbers indicate the number of rats exhibiting the finding over the number of rats examined.

Table 2B
Summary of Clinical Observations*
Multidose Range-Finding Study in Rats - Multidose Range-Finding Study
Males

OBSERVATION	Dose 1 Day 1 Hours Postdose 1	5	Dose 2 Day 2 Hours Postdose 1	6	Dose 3 Day 3 Hours Postdose 1	6	Dose 4 Day 4 Hours Postdose 1	6	24
Group 1 - 500 mg/kg									
Normal	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Group 2 - 1000 mg/kg									
Normal	1/2	1/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Soft feces	1/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Group 3 - 2500 mg/kg									
Normal	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Depression	1/2	0/2	1/2	0/2	1/2	0/2	0/2	0/2	0/2
Slight depression	1/2	1/2	1/2	1/2	1/2	1/2	0/2	0/2	0/2
Rough coat	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Soft feces	0/2	0/2	1/2	0/2	1/2	1/2	0/2	0/2	0/2
Urine stains	2/2	2/2	0/2	1/2	0/2	1/2	0/2	0/2	0/2

* Numbers indicate the number of rats exhibiting the finding over the number of rats examined.

Table 2B - Continued
Summary of Clinical Observations*
Multidose Range-Finding Study in Rats - Multidose Range-Finding Study
Females

OBSERVATION	Dose 1 Day 1 Hours Postdose 1	Dose 1 Day 1 Hours Postdose 5	Dose 2 Day 2 Hours Postdose 1	Dose 2 Day 2 Hours Postdose 6	Dose 3 Day 3 Hours Postdose 1	Dose 3 Day 3 Hours Postdose 6	Dose 4 Day 4 Hours Postdose 1	Dose 4 Day 4 Hours Postdose 6	Dose 4 Day 4 Hours Postdose 24
Group 1 - 500 mg/kg									
Normal	2/2	2/2	2/2	1/2	1/2	2/2	2/2	2/2	2/2
Urine stains	0/2	0/2	0/2	1/2	1/2	0/2	0/2	0/2	0/2
Group 2 - 1000 mg/kg									
Normal	1/2	1/2	2/2	1/2	2/2	2/2	2/2	2/2	2/2
Urine stains	1/2	1/2	0/2	1/2	0/2	0/2	0/2	0/2	0/2
Group 3 - 2500 mg/kg									
Normal	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Depression	0/2	0/2	1/2	0/2	1/2	0/2	0/2	0/2	0/2
Slight depression	2/2	1/2	1/2	2/2	1/2	2/2	0/2	0/2	0/2
Ataxia	0/2	0/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2
Rough coat	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Soft feces	1/2	1/2	1/2	0/2	1/2	0/2	0/2	0/2	0/2
Urine stains	1/2	1/2	2/2	1/2	2/2	1/2	1/2	2/2	0/2

* Numbers indicate the number of rats exhibiting the finding over the number of rats examined.

Table 3A
Initial Body Weights
Multidose Range-Finding Study in Rats
Single Dose Range-Finding Study

Animal Number	Sex	Initial Body Weight (grams)
	Group 1 - 5000 mg/kg	
D96747	M	154.3
D96748	M	162.4
D96753	F	136.4
D96754	F	117.4

Table 3B
Initial Body Weights
Multidose Range-Finding Study in Rats
Multidose Range-Finding Study

Animal Number	Sex	Initial Body Weight (grams)
	Group 1 - 500 mg/kg	
D96983	M	182.4
D96984	M	224.1
D96995	F	133.3
D96996	F	146.2
	Group 2 - 1000 mg/kg	
D96985	M	186.4
D96986	M	193.3
D96997	F	156.5
D96998	F	131.6
	Group 3 - 2500 mg/kg	
D96987	M	204.3
D96988	M	223.0
D96999	F	148.0
D97000	F	124.3